COMPLEMENT FIXATION. IN INFLUENZA WITH B. INFLUENZAE ANTIGENS

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During the past two or three years some doubt has arisen regarding the part played by B. influenzae as an etiologic agent in influenza. It is agreed that the organism is almost universally present in the upper respiratory secretions of patients with the disease, and also that there are large numbers of bacillus carriers both in healthy individuals and in those suffering from other respiratory infections. From this widespread distribution of the influenza bacillus, therefore, has arisen the question whether the organisms found in influenza patients are present as infecting parasites, or merely as relatively harmless saprophytes. If parasitic, they may be the cause of the disease or may be present as secondary invaders, although the latter seems somewhat less likely. Since one of the evidences of infection by an organism is the production of specific antibodies, some effort has been made to determine the presence of specific antibodies to the influenza bacillus in the serums of influenza patients. The presence of such specific substances would supply indirect evidence of the etiologic relation of the organism to influenza. This article gives the results of a study of serums from influenza patients by the complement fixation test using several strains of B. influenzae as antigens.

Several investigators have recently studied the complement fixing substances in influenza serums, but all have not obtained uniform results.

The largest series of cases was reported by Rapoport,[1] who found 54.5% of positive fixation in 295 serums from patients convalescing from influenzal pneumonia, using influenza bacillus antigens. Some serums were positive 41 days after convalescence began, but most of the positive reactions were obtained on patients convalescent 3 to 4 days. Durand[2] with an influenza bacillus antigen obtained negative fixations in 5 cases of uncomplicated influenza during convalescence, and 2 questionably positive reactions in 4 patients with pneumonia. Four other cases in which B. influenzae was found in the nasopharynx gave 2 positive and 1 questionable fixation. Kolmer, Trist and Yagle,[3] found 45 to

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[1] Jour. Am. Med. Assn., 1919, 72 p. 633.
[2] Riforma Med., 1919, 35, p. 458.
[3] Jour. Infect. Dis., 1919, 24, p. 583.

51% of positive fixations in 31 cases of convalescent influenza with Pfeiffer bacillus antigens, while 38% of the same serums were positive with both the hemolytic streptococcus, and M. catarrhalis antigens in addition. Reactions using staphylococcus and a pseudodiphtheria bacillus antigen were negative except one serum which reacted with the staphylococcus. Nine healthy controls gave negative fixation tests with all antigens. Howell and Anderson[4] obtained only 20% of positive fixations in 253 tests in 59 cases of influenza by the use of B. influenzae antigens, while they obtained many more positive results on serums from the same patients using as antigen certain strains of the viridans group of streptococci.

Gay and Harris,[5] using a polyvalent influenza antigen, obtained positive fixation in only one of 29 acute cases of influenza, but in 40% of 25 vaccinated patients from 2 to 37 days after the last injection. The serum of rabbits immunized with mixed cultures of B. influenzae gave fixing antibodies in high dilutions. Wollstein[6] examined 5 mild cases and 7 complicated by pneumonia, and obtained positive fixations in all and negative reactions in 4 normal adults. The cases were examined from the sixth to the twenty-third day of the disease, and stronger reactions were obtained as convalescence advanced. Eight different strains of Pfeiffer's bacillus were used as antigen, and some serums reacted with only one strain while other serums gave fixations with all strains tested. In 19 persons who had recovered from influenza from 1 to 4 months previously, irregular results were obtained, but some serums showed the presence of fixing antibodies. Rabbits immunized with 10 strains of influenza bacilli gave positive fixations with both homologous and heterologous strains. Fry and Lundie,[7] using a salt solution extract of one strain of B. influenzae, found 6 of 8 cases of influenza gave positive fixation reactions, while 32 controls showed one positive and 5 weakly positive or doubtful reactions.

In this paper are given the results of complement fixation tests in children and adults with influenza pneumonia, using several strains of B. influenzae as antigens.

METHODS

The strains of B. influenzae used were isolated by Dr. H. H. Bell and their source and characteristics described by him.[8] In preparation of the antigens, a 24-hour growth on blood-agar plates was washed off with salt solution to form an emulsion of moderate opacity. This was heated to 65 C. for one hour, and titrated to determine its anticomplementary properties. Each antigen was then diluted with salt solution so that half the largest amount, which was not at all anticomplementary, was contained in 0.1 c c and this antigen dose used in all tests. Usually a dilution of 4 or 5 times was required.

The patients' serums were inactivated for 30 minutes at 56 C. and 0.1 c c, 0.05 c c, and 0.01 c c used for each fixation test.

[4] Jour. Infect. Dis., 1919, 25, p. 1.
[5] Jour. Infect. Dis., 1919, 25, p. 414.
[6] Jour. Exper. Med., 1919, 30, p. 555.
[7] Lancet, 1920, 198 p. 368.
[8] Jour. Infect. Dis., 1920.

The hemolytic system consisted of two units of complement with 0.5 c c of 1% suspension of sheep corpuscles sensitized with 2 units of hemolysin. The antigen, serum and complement were incubated 1 hour after the addition of the sensitized cells. The total volume of each test was 2.5 c c, and the readings made after standing over night in the icebox. Details of the method used may be found elsewhere.[9] The usual controls of serum without antigen and of twice the amount of antigen used were always included.

The considerable dilution of antigen required to overcome its anticomplementary power may have interfered in certain cases with its antigenic properties. However, since all antigens except one gave fixations with some serums, they were probably antigenic in the amounts used. No attempt was made to prepare a permanent antigen that could be used throughout the entire series of tests. The tests were carried out at various times and on each occasion the antigens were prepared in the same manner so that it is likely that the results of tests made at different times are for the most part comparable.

All possible cross-fixations could not be done on account of the lack of sufficient serum from every case. Each serum was tested with from 4 to 12 antigens and in some cases the tests were repeated with another specimen of serum taken at a later date. In order to control to a certain extent the specificity of the antigens, all serums were also tested with a human tubercle bacillus antigen and all gave negative results.

Twenty-one of the cases tested were uncomplicated, and had typical clinical symptoms with fever and leukopenia. In 14, bronchopneumonia of varying severity prolonged the illness but was fatal in only one instance. The blood in all cases, except the fatal one, was obtained after the temperature had become normal, usually in the second week after the onset of the first symptoms. All were patients in the Barnes and St. Louis Children's Hospitals during the epidemic of the early months of 1920. Organisms morphologically and culturally resembling B. influenzae were isolated from the sputum or the nasopharyngeal secretions of a number of the patients by Dr. H. H. Bell, Dr. Park J. White, and Dr. A. M. Chesney.

On account of the wide prevalence of the disease and the almost certain occurrence of many unrecognized mild infections, it was difficult to secure many suitable control serums from persons known to have escaped the disease. Four children and 9 adults (medical stu-

[9] Cooke, J. V.: Jour. Infect. Dis., 1919, 25, p. 452.

dents), none of whom had had any clinical symptoms of influenza, were selected as examples of probably uninfected persons and their serums used as controls.

In tabulating the results of the tests, a division has been made into several groups. Children under 6 years are shown in table 1, while the older children are given in table 2. This separation was made on account of an observation on complement fixation for tuberculosis in children.[10] Here it was found that tuberculous children under 6 years of age gave a much smaller proportion of positive results than the older children, while the percentage of the latter giving positive reactions was quite similar to that found in adults. In table 3, the tests on adults with influenza are collected, and in

TABLE 1
COMPLEMENT FIXATION TESTS IN INFLUENZA IN CHILDREN UNDER 6 YEARS

	Case Number										
	1*	2*	3*	4*	5	6*	7	8	9	10*	11
Day of disease	8	7	21	7	7	12	4	8	11	8	15
Age in years	1†	1†	1†	2	2	2	3	3	4	4	4
Antigens:											
4. Cotton	..	0	++	0	..	..	0	0	..	0	
5. B. Ouslander	..	..	..	..	..	..	++				
6. 198	+	0	..	0	0	0	0	0	++	..	++
12. Lasersohn	+	±	++	0	0	0	0	0	0	0	0
13. Ferguson	0	++	+	0	0	0	++	+	++	0	++
14. Stolte	0	..	0	0	0	0	0	0	0	0	+
15. 257	..	..	0	++	0	..	..	0	..	0	+
16. Parsons	0	..	..	..	0	0	..	..	0	..	++

* B. influenzae isolated from pharynx.
† Influenza with pneumonia.

TABLE 2
COMPLEMENT FIXATION TESTS IN INFLUENZA IN CHILDREN 6 TO 14 YEARS

	Case Number												
	12*	13	14*	15*	16*	17	18	19*	20*	21	22	23*	24*
Day of disease	7	14	7	7	14	6	12	16	14	30	28	12	30
Age in years	6†	6†	6†	6	7†	8	9†	9	10†	10	11†	13†	13†
Antigens:													
1. Essermann	++	..	..	++	..	..	..	..	..	..	..	++	
2. Smith	0	..	..	0	..	..	..	..	..	..	..		
3. 223	++	..	..	++	..	..	..	..	..	..	..	++	
4. Cotton	++	..	±	±	±	+	++	..	..	..	0	±	
5. B. Ouslander	++	..	++	++	++	..	..	..	..	..	..	++	0
6. 198	++	..	++	++	++	..	++	++	++	+	++	++	±
7. 240	++	..	++	±	++	..	..	..	..	..	..	++	
8. Bell	++	..	..	++	..	..	..	..	..	..	0	++	++
9. R. Ouslander	++	..	..	++	..	..	..	..	..	..	..	++	
10. 226	++	..	..	++	..	..	..	..	..	..	++	+	0
11. Dewey	++	..	..	++	..	.	..	..	..	..	..	±	
12. Lasersohn	..	++	++	..	..	±	++	0	++	+	0	..	0
13. Ferguson	..	++	++	..	..	+	++	++	++	0	++	..	++
14. Stolte	..	0	++	..	..	++	++	±	0	0	0	..	0
15. 257	..	..	++	..	..	+	++	0	..	..	0	..	0
16. Parsons	..	++	0	..	0	..	..	++	0	0	0	..	++

* B. influenzae isolated from pharynx.
† Influenza with pneumonia.

[10] Cooke, J. V.: Am. Jour. Dis. Child. In press.

TABLE 3
COMPLEMENT FIXATION TESTS IN INFLUENZA — ADULTS

	Case Number										
	25	26*	27*	28*	29*	30*	31*	32	33*	34*	35*
Day of disease	30	13	13	10	14	10	10†	7†	14	10	10
Antigens:											
1. Essermann	..	..	++	..	..	++					
2. Smith	..	..	0	..	..	0					
3. 223	..	..	++	..	..	++					
4. Cotton	±	0	±	..	±	±	±	++	++	..	+
5. B. Auslander	..	++	++	++	..	++	++	..	..	++	++
6. 198	..	..	++	..	0	++	++	0	++		
7. 240	..	..	++	++	..	±	++	..	..	+	
8. Bell	..	..	++	..	..	++	..	..	..	..	++
9. R. Auslander	..	..	++	++							
10. 226	..	..	++	..	..	++	..	..	..	++	++
11. Dewey	..	..	++	++	..	++	..	..	..	..	±
12. Lasersohn	++	0	..	..	0	..	±	±	+		
13. Ferguson	++	0	..	..	++	..	±	±	++		
14. Stolte	±	0	..	..	..	..	0				
15. 257	0	++	..	..	..	..	0				
16. Parsons	0	..	..	..	..	..	0				

* B. influenzae isolated from sputum or from pharynx.
† Influenza with pneumonia.

TABLE 4
COMPLEMENT FIXATION TESTS ON CHILDREN AND ADULTS WITHOUT CLINICAL INFLUENZA

	Case Number												
	36	37	38	39	40	41	42	43	44	45	46*	47	48
Age in years	¼	3	5	8	A	A	A	A	A	A	A	A	A
Antigens:													
1. Essermann	..	0	0	..	..	..	0	..	..	..	0	..	0
2. Smith	..	0	..	..	0								
3. 223	0	0	0	..	..	..	0	..	..	..	0	..	0
4. Cotton	..	0	0	0	0	0	0	0	0	0	0	0	0
5. B. Auslander	0	..	0	0	..	..	..	..	..	0			
6. 198	..	0	0	0	0	0	0	0	++	±	0	0	0
7. 240	..	..	..	0	0								
8. Bell	..	..	..	..	..	0	0	0	++	++	++	++	++
9. R. Auslander	..	..	..	0	0	0	..	..	++	..	..	+	
10. 226	..	..	..	..	..	0	0	0	0	0	0	0	0
11. Dewey	0	..	0	..	0	..	..	0					
12. Lasersohn	0	0	0	0	0	0	0	0	0	0	0	0	0
13. Ferguson	0	±	0	0	0	0	±	0	0	0	++	0	++
14. Stolte	0	..	..	..	..	0	0	0	0	0	0	0	++
15. 257	..	..	..	..	..	0	0	0	0	0	0	0	0
16. Parsons	0	..	..	..	..	0	±	++	±	++	++	++	++

* B. influenzae isolated from pharynx.

table 4, the control tests. In indicating the strength of fixation found in the different serums, "++" shows complete fixation with 0.5 c c or less of serum; "+," complete fixation with 0.1 c c only, and "±," partial fixation with 0.1 c c. A "O" means no fixation with 0.1 c c and a blank indicates that the test was not made.

Eleven younger children, between 1 and 4 years old were tested with 8 strains of B. influenzae as antigen (table 1). While fixation was noted in all but 3 of the serums, with one or more antigens, the results were irregular. There was no uniform fixation with any of

the serums nor was any antigen constant in its fixing properties. Of the 59 tests, only 17 were positive, 6 of these being weak reactions.

With the older children and adults (tables 2 and 3) the presence of fixing bodies was much more evident, although in these also some irregularity was noted. Certain antigens fixed well with almost all cases tested, while other strains failed to give fixation with some of the serums. No order could be detected in this irregularity nor could any grouping of the antigens be made, since some antigens that gave good fixation with some of the serums, failed to fix in other instances in which other antigens had given positive results. In all 162 tests were made on 24 serums in these 2 groups using 16 strains of B. influenzae, and more than 60% of the reactions were positive. All serums tested gave fixation with two or more of the antigens, and only one of the antigens failed to give fixation with any of the serums. The patients with pneumonia as a rule gave good fixation, but in this group no better results were noted than those that did not develop this complication.

The controls included 13 cases (table 4) and of the 120 tests done, 16% were positive. These positive reactions were obtained in 6 of the serums with 6 of the antigens, one of the positive tests being given by the serum of an apparently healthy carrier. The explanation of the positive reactions obtained in certain of the controls is not clear, but the possibility of a previous unrecognized infection in these persons is difficult to exclude. By comparing the tables, however, there is a striking difference noted in the number of positive reactions obtained in the groups studied. Many more positive reactions were found in the older children and adults with influenza than in the group of younger children, while the control group showed the smallest number of positive reactions.

CONCLUSIONS

Complement fixing antibodies can be demonstrated in the serum of a considerable number of older children and adults convalescent from influenza by the use of B. influenzae antigens. These antibodies are much less constantly found in children from 1 to 5 years of age. No definite antigenic relationship could be detected between the 16 strains of B. influenzae with the serums tested. The results indicate that the influenza bacillus is pathogenic and infects many, if not all, patients with influenza. The complement fixation test cannot furnish sufficient evidence, however, to justify the conclusion that B. influenzae is the sole etiologic agent in influenza.